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Bisphenol A degradation by *Penicillium* sp isolated from Agro Industry Effluent

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ABSTRACT
Bisphenol A (BPA) is commonly suspected to act as an endocrine disrupter. Due to increase in the use of products based on epoxy resins and polycarbonate plastics, exposure of humans to BPA through several routes to the environment. Hence, it is necessary to remove Bisphenol A from industrial effluents before discharging them into the environment. This study examined the biodegradation of BPA by *Penicillium* sp isolated form agro industry effluent. Physiochemical values of the effluent are studied and reported. The method of batch experiment was used for the degradation study by using BPA at different concentrations ranged from 20-100ppm. The highest removal of 80% was found in 20ppm. The effect of pH like 5.0 to 11.0 on growth of fungi strain and BPA removal was examined. This study was the preliminary work to access the degradation efficiency of *Penicillium* sp which was isolated from the agro industry effluent.

Keywords: Bisphenol A, *Penicillium* sp, effluent, isolation, degradation

INTRODUCTION

The global growth and rapid industrial development have led to the recognition and increasing understanding of interrelationship between pollution, public health and environment. While almost industrial activities cause some pollution and produce waste, relatively few industries without pollution control and waste treatment facilities are responsible for the bulk of the pollution [1]. In most developing countries like India, most industries dispose their effluents without treatment. Wastewaters generated by the chemical, petrochemical and steel industries, frequently contain high concentrations of phenolic compounds, which present a serious ecological problems due to their widespread use, toxicity and occurrence in the environment [2].

Phenolic compounds are common waste by products in the manufacture of industrial and agricultural products. Bisphenol A [BPA; 2, 2-bis (4-hydroxyphenyl) propane] is an industrially important compound. It is one of the materials necessary for the production of polycarbonates, epoxy resins and other plastics, and its worldwide annual consumption is increasing. BPA is strongly suspected to be an endocrine disruptor [3]. Furthermore, it was reported that BPA has estrogenic activity [4], and demonstrated developmental and reproductive toxicities in rats and mice fed at high doses [5], with particularly strong effects in foetus [6-8].

Microbial degradation is expected to play a major role in the removal of BPA from the environment. Rapid and extensive breakdown of BPA has been demonstrated in a variety of laboratory biodegradation tests [9]. Reports showed BPA degrading microorganism, strain MV1, was isolated from sludge taken from the wastewater treatment plant at a plastic manufacturing facility [10]. BPA was metabolized by strain MV1 via a novel pathway involving oxidative skeletal rearrangement [11]. *Sphingomonas paucimobilis* strain FJ-4 was isolated from an activated sludge taken from the wastewater treatment plant at an epoxy resin manufacturing facility [12]. Recent studies indicate that BPA is degraded rapidly in surface water and sediments taken from a wide variety of geographies, suggesting that microorganisms with the capability to degrade BPA are ubiquitous in the environment [13].

In the present paper we report the isolation of *Penicillium* species form agro industry effluent and their biodegradation efficiency of Bisphenol A at various concentrations.

MATERIALS AND METHODS

Sample collection

Effluent samples were collected from agro industry located in Erode district, Tamilnadu, India. The physicochemical quality of the raw effluent was analysed [14]. The results are expressed in mg/L for effluents and receiving water.

Isolation, Screening and Growth characteristics

Isolation of fungi was carried out on Czapekdox agar and Potato Dextrose agar (PDA) following serial dilution technique. The agar plates were incubated at 21^0C for 7 days. Morphologically distinct colonies were subjected to purification following sub culturing. The pure cultures were maintained on PDA slants at 4 ^{0}C in a refrigerator. The isolated fungal strains were tested for their independent tolerance of BPA in Czapekdox agar medium both in broth and plates. Filter sterilized BPA at different concentrations were added to the medium after autoclaving. Strains were inoculated and observed for growth after 5 days at 28ºC. Out of tolerant strains one strain was chosen for the study.

Fourier Transform Infrared Analysis

The functional groups present in the fungi used for this study was examined using Fourier Transform Infra Red (FTIR) spectroscopy. The spectra of the sample were recorded between 4000 and 400 cm^{-1} using thermo Nicolet, Avatar 370 spectrometer (Model: IR Affinity brand, Shimadzu) at Karpagam University, Coimbatore.

Biodegradation of BPA by batch experiment

The tolerant strain was aseptically inoculated (2 ml. of fresh spore suspension of inoculums strength: 5x 101 c.f.u./ml) in 100 ml of sterile distilled water supplemented with BPA of varied (20,40,60,80 and100ppm) concentrations in 500 ml glass flasks. Control sets without fungus were also run. The experimental set up was carried out in orbital shaker at room temperature to study the ability of the strain capable of assimilating BPA as sole carbon source. The organism growth rate and BPA removal efficiency were calculated by optical density at 270nm for each day interval up to 5^{th} day. BPA estimation was done with filtrate after harvesting fungal biomass by centrifugation at 10,000 rpm for 15 min. Effect of pH on the degradation process was tested by addition of fungi strain into 20ppm of BPA solution prepared with different pH (3.0, 5.0, 7.0, 9.0 and 11.0) levels.

Statistical analysis

Mean triplicate readings obtained in the study were subjected to analysis of variance (ANOVA) and Ducan's multiple range tests using statistical package for Social Scientist (SPSS 10.0) computer software [15].

RESULTS AND DISCUSSION

In the present study the agro industry effluents were used as source for fungal strain isolation for the biodegradation of BPA. The physicochemical quality of the collected effluent was tested and reported (Table 1). Out of few tolerant fungal strains one of the most frequently occurring genus was observed throughout the isolation procedure and it was identified as *Penicillium* based on colony morphology and microscopic observations [17, 18]. Many studies have found that BPA is degraded in the environment, as in activated sludge [9, 10], river water [19, 20] and soil [21, 22] and that some BPA degrading micro organisms were isolated from such places, but not from agro industry effluent.

FTIR spectra were analyzed using a revised algorithm for baselining and spectral smoothing, fitting overlapping peaks, and integrating absorbance before converting to mass with standard calibrations. FTIR spectromicroscopy can provide spatially resolved biochemical information on hyphal growth and spore development under normal and stressful conditions, complementing information gleaned from molecular genetics investigations [23]. In FTIR analysis major peaks were observed at 3373.5 cm^{-1} which may be polymeric hydroxyl compounds due to O-H Stretching (bonded) functional group, 2929.87 cm^{-1} may be Cyclobutane due to C-H stretching and 1739.79 cm^{-1} which may be Aliphatic aldehyde due to C=O stretching. The peaks were observed at 1647.21 cm^{-1} may be Polyene due to C=C stretching , 1076.28 cm^{-1} which may be alkyl amine due to C-N stretching and 1039.63 cm^{-1} may be acid and RSO_3 , ionic Sulphonates due to the presence of SO_3 symmetric stretching (Figure 1).

Table 1 The physicochemical quality of agro industry effluents (mg/l)

Parameters	Effluent
pH	5.60±0.18
Dissolved oxygen	25.00±0.70
BOD	186.00±5.15
COD	133.00±3.85
Conductivity	18.00±0.60
Hardness	56.00±1.69
Turbidity	98.40±2.89
Dissolved solids	800.00±24.09
Total solids	2200.00±74.51

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Values are means of triplicate readings ± SEM

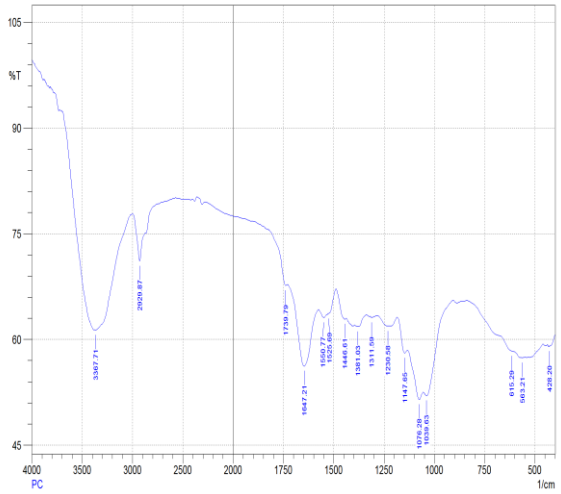

Figure 1. FTIR analysis of selected *Penicillium* sp

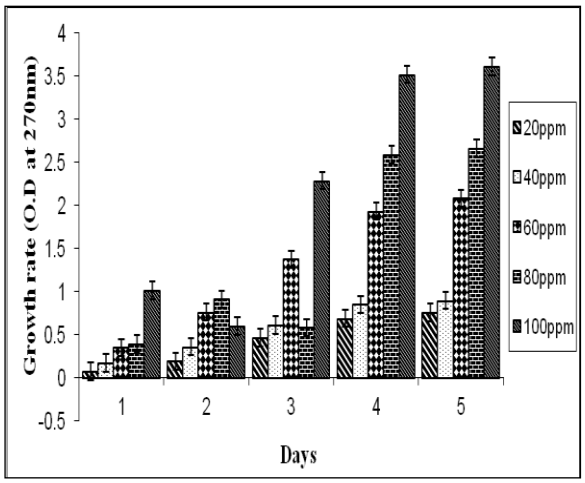

Figure 2. Growth rate of *Penicillium* sp in different concentrations of BPA

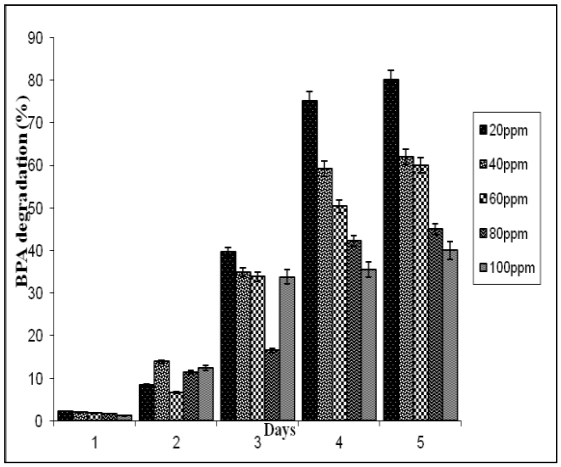

Figure 3. BPA degradation efficiency by *Penicillium* sp

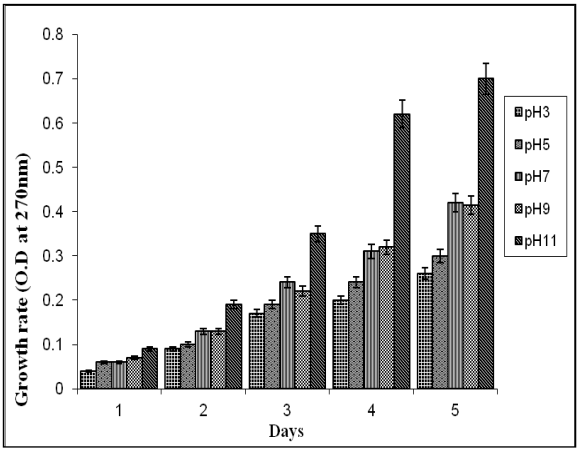

Figure 4. Effect of pH on *Penicillium* sp growth in 20ppm BPA solution

The growth rate of *Penicillium* sp in different concentration ranges from 20ppm to 100ppm was measured for 5 days and are reported (Figure 2).

BPA degradation by 26 strains of soil fungi were examined in earlier studies [24]. Among the 26 strains tested, 22 showed good growth for this initial concentration of BPA. After 14 day of reaction, these strains were grown to about 60 mg (dry wt) and 11 strains degraded BPA at 50% or higher (Chai *et al*., 2005). In this study the highest degradation (>99%) was found in *Aspergillus*, with *A. terreus* MT-13; *Fusarium*, with *F. moniliforme* 2-2 and *F. sporotrichioides* NFRI-1012; and *Ascomycetes*, with *Emericella nidulans* MT-78. In the present work *Penicillium* sp was used for degradation and showed the growth in all concentration up to 100ppm. Maximum removal of 80% degradation was at 20ppm BPA solution and at 100ppm 50% removal obtained (Figure 3).

The effect of pH for different fungal strains observed that BPA degradation was obtained at the level of pH 11.0 in previous study [24]. In the present study effect of pH on the growth of *Penicillium* sp in synthetic BPA solutions at 20ppm with various pH ranged from 3.0 to 11.0. The data stated that the highest cell density and degradation were obtained when pH is 11.0, indicating pH preference of the fungi (Figure 4).

In the investigations that are conducted with a view of understanding the microbial diversity and the associated biotechnological applications, after having a maximum number of isolates from a given environment, a rapid screening for obtaining the efficient strains with desired character is a prerequisite. However further studies are needed to understand the clear mechanism involved in this degradation process. degradation process was investigated, and it showed fungal biomass were dependent on both factors. This study showed the preliminary aspects of isolated *Penicillium* sp in biodegradation of BPA. Furthermore, Studies are needed to examine the clear degradation pathway.

CONCLUSION

The agro industry effluents were used as a source of isolation fungi for the biodegradation of Bisphenol A. The fungi strain which most frequently occurring throughout the isolation process was selected and identified as *Penicillium* based on colony morphology and microscopic observations. The functional groups present in the fungi were examined using FTIR analysis. *Penicillium* sp was inoculated the different BPA concentration like a 20 to 100ppm. The growth increased rapidly all concentrations at the initial period and slowly towards the equilibrium level at 5^{th} day. Optimum pH range identified by using different pH ranged from 3.0 to 11.0 in 20ppm of BPA solution. The present investigation reported that *Penicillium* sp utilize the BPA as a carbon source for their growth. The effect of pH and time for the growth of fungi and

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